Clinical Infectious Diseases

MAJOR ARTICLE

Early Vaccination With Bacille Calmette-Guérin-Denmark or BCG-Japan Versus BCG-Russia to Healthy Newborns in Guinea-Bissau: A Randomized Controlled Trial

Frederik Schaltz-Buchholzer,[1,2,3] Morten Bjerregaard-Andersen,[2,3] Christian Bjerregård Øland,[3] Christian Golding,[2,3] Elise Brenno Stjernholm,[2,3] Ivan Monteiro,[2] Peter Aaby,[2] and Christine Stabell Benn[1,2,3,4]

[1]Institute of Clinical Research, University of Southern Denmark and Odense University Hospital, Odense, Denmark, [2]Bandim Health Project, INDEPTH Network, Bissau, Guinea-Bissau, [3]Research Center for Vitamins and Vaccines, Statens Serum Institut, Copenhagen, Denmark, and [4]Danish Institute of Advanced Science, University of Southern Denmark, Odense, Denmark

(See the Editorial Commentary by Danchuk and Behr on pages 1894–5.)

Background. Bacille Calmette-Guérin (BCG) vaccination remains a cornerstone against tuberculosis. Randomized controlled trials (RCTs) have demonstrated that BCG-Denmark lowers all-cause mortality, but a recent RCT found no effect of BCG-Russia. Observational studies indicate that the genetically divergent BCG strains have different effects.

Methods. This was a parallel-group, open-label RCT conducted at the National Hospital in Guinea-Bissau. Healthy neonates were randomized 1:1 to BCG-Denmark (2851 randomized, 2840 analyzed) vs BCG-Russia (2845 randomized, 2837 analyzed). We hypothesized that BCG-Denmark would reduce morbidity (primary outcome) and mortality while inducing more BCG reactions and purified protein derivative (PPD) responses (secondary outcomes). Halfway through the trial, production of BCG-Denmark was halted, and the trial continued comparing BCG-Japan (3191 neonates randomized, 3184 analyzed) with BCG-Russia (3170 randomized, 3160 analyzed). Mortality and morbidity data were collected by telephone, at home visits, and at the National Hospital and assessed in Cox models providing 6-week mortality rate ratios (MRRs) and hospitalization incidence rate ratios (IRRs).

Results. By age 6 weeks, there were 140 and 130 admissions among neonates vaccinated with BCG-Denmark and BCG-Russia, respectively (IRR, 1.08 [95% confidence interval {CI}, .84–1.37]). For BCG-Japan, there were 185 admissions vs 161 admissions for BCG-Russia (IRR, 1.15 [95% CI, .93–1.43]). The 6-week mortality did not differ: BCG-Denmark/BCG-Russia (MRR, 1.15 [95% CI, .74–1.80]); BCG-Japan/BCG-Russia (MRR, 0.71 [95% CI, .43–1.19]). BCG-Denmark and BCG-Japan induced more BCG scars and PPD reactions than BCG-Russia.

Conclusions. BCG strains did not affect morbidity. BCG-Denmark and BCG-Japan were more immunogenic than BCG-Russia by the measures traditionally viewed as surrogates for successful immunization. The implications of strain differences for tuberculosis protection and overall health warrant further study.

Clinical Trials Registration. NCT02447536.

Keywords. BCG strains; early-life morbidity and mortality; tuberculosis; nonspecific effects of vaccines; purified protein derivative response.

Bacille Calmette-Guérin (BCG) vaccine has been administered against tuberculosis (TB) for almost a century. A range of different live attenuated BCG vaccine strains are used worldwide [1]. BCG provides variable protection against pulmonary TB and good protection against miliary and meningeal TB [2]. Besides the disease-specific effects, there is accumulating evidence that BCG reduces all-cause mortality. In a meta-analysis of 3 randomized controlled trials (RCTs) that provided early BCG-Denmark and oral polio vaccine (OPV) vs OPV only, BCG-Denmark reduced all-cause neonatal mortality by 38% (95% confidence interval [CI], 17%–54%) [3]. These effects are much larger than can be ascribed to prevention of TB, indicating that BCG has beneficial nonspecific effects (NSEs) [4]. Epigenetic modifications in innate immune cells, so-called trained immunity, have been suggested as an immunological mechanism [5].

Several diverse BCG strains exist because the sensitive live mycobacteria have accumulated substantial genetic divergence during decades of reproductive cycles in different laboratories, but little is known regarding their relative effects and potencies [2, 6]. The RCT data from Guinea-Bissau are in contrast to a recent Indian RCT that reported no effect of early BCG-Russia, the neonatal BCG/no-BCG hazard ratio being 0.98 (95% CI, .85–1.11) [7]. It has been reported that BCG strains

Received 22 July 2019; editorial decision 28 September 2019; accepted 29 October 2019; published online November 3, 2019.

Correspondence: F. Schaltz-Buchholzer, Bandim Health Project, Studiestræde 6, 1455 Copenhagen, Denmark (fsbr@ssi.dk).

Clinical Infectious Diseases® 2020;71(8):1883–93

DOI: 10.1093/cid/ciz1080

differ by a range of factors including their adverse event profiles and their ability to induce scars and purified protein derivative (PPD) reactions [8–10]. BCG's immunogenicity has traditionally been evaluated by these factors and it is well-established that a dose-response correlation exists between the amount of viable colony-forming units (CFUs) injected at vaccination and the successful induction of BCG scars and PPD reactivity [11]. BCG's beneficial NSEs on overall survival are enhanced among vaccinated infants who develop a scar and/or reactivity to PPD compared to vaccinated infants who do not develop a scar/PPD response [12–17].

Despite an accumulated 4 billion BCG-vaccinated humans and >100 million annual vaccinations, only a few RCTs have compared now-obsolete strains, and a review did not find evidence to favor a particular strain [2]. The currently World Health Organization–prequalified strains used by the United Nations Children's Fund have never been tested head-to-head in an RCT. We initiated an RCT to evaluate both specific (BCG reactions and PPD responses) and nonspecific (all-cause morbidity and mortality by 6 weeks of age) outcomes among major BCG strains.

METHODS

Setting

The Bandim Health Project (BHP; www.bandim.org) maintains a Health and Demographic Surveillance System (HDSS) covering approximately 100 000 inhabitants in Bissau, the capital of Guinea-Bissau, a low-income country with high neonatal and infant mortality. The trial took place at the Simão Mendes National Hospital (HNSM) maternity ward, which is the country's principal birthplace (6000–7000 deliveries/year). BHP staff document all births and vaccinations at the ward and all neonates receive BCG and OPV on the day of discharge; a series of other infant vaccines are provided at smaller health centers from 6 weeks of age. HNSM also hosts the only specialized pediatric ward in Guinea-Bissau, where BHP staff document admissions and their outcomes [18].

Study Design

The BHP initiated this hospital-based, open-label, parallel-group RCT in December 2014 aiming to recruit 12 000 neonates randomized individually 1:1 to BCG-Denmark (intervention) or BCG-Russia (controls). A production halt at the manufacturing unit of Statens Serum Institut (SSI) in Copenhagen meant that BCG-Denmark became unavailable after mid-2015. By July 2016, our last stock of BCG-Denmark expired. Prior to this date, permissions had been granted from the data and safety monitoring board and the ethical committee to replace BCG-Denmark with BCG-Japan for the remainder of the trial.

Enrollment and Informed Consent

Healthy neonates born at the HNSM with no severe malformations were eligible and invited to participate on the day of hospital discharge. Mothers/guardians were provided written study information in Portuguese and an oral explanation of the study in the local language Creole, as well as the opportunity to ask questions. Most participants were recruited within a few days after birth (Table 1). At enrollment, we collected maternal socioeconomic data such as ethnicity, age, residential area, and available telephone contacts for parents and relatives. We recorded the maternal mid-upper-arm circumference (Supplementary Figure 1) and BCG scar status as well as birth weight, inclusion weight, and twinning status for the neonate. A detailed specification of randomization procedures is provided in the Supplementary Appendix.

Interventions

In phase 1 of the RCT, neonates were randomized 1:1 to BCG-Denmark (Copenhagen strain 1331, SSI) or BCG-Russia (Russia BCG-I strain, Serum Institute of India). In phase 2 of the RCT, neonates were randomized 1:1 to BCG-Japan (Tokyo strain 172, Japan BCG Laboratory) or BCG-Russia (Russia BCG-I strain, Serum Institute of India). Two vaccinators with >15 years of experience performed all study vaccinations by injecting 0.05 mL of reconstituted BCG intradermally in the left deltoid region (Supplementary Figure 2), followed by OPV administration.

Follow-up

Information on outcomes was obtained from data collection at HNSM, through telephone interviews, and at HDSS home visits.

Hospital Registration and Follow-up

HNSM hosts the only specialized pediatric ward in Guinea-Bissau, where BHP staff document admissions and their outcomes [18] (Supplementary Figure 3). We applied a standardized data-linkage protocol to identify all study participant admissions in the pediatric ward database (Supplementary Appendix).

Telephone Follow-up

We conducted telephone interviews at 6 weeks of age collecting mortality data from families with a telephone number registered at inclusion. Priority was given to interview the mother when possible. Available contacts were telephoned 3 times on 3 separate days to ensure a high follow-up rate.

Home Visit Follow-up

For enrolled infants from our HDSS, a field assistant blinded to randomization allocation conducted standardized visits at 2 and 6 months of age to collect anthropometric data, review the child's skin BCG reaction status, evaluate the PPD response

Table 1. Baseline Inclusion Characteristics for Intervention and Control Children, by Study Phase

Characteristic	Phase 1: Before 1 July 2016		Phase 2: From 1 July 2016	
	BCG-Denmark	BCG-Russia	BCG-Japan	BCG-Russia
Included, No. (%)	2840 (50)	2837 (50)	3184 (50)	3160 (50)
Maternal characteristics				
Mother has BCG scar[a]	58% (1256/2159)	59% (1265/2156)	60% (1896/3151)	60% (1892/3132)
Median BCG scar size, mm (IQR)	7 (5–9)	7 (5–9)	7 (5–9)	7 (5–9)
Median maternal age, y (IQR)	25 (20–29)	25 (21–30)	25 (21–30)	25 (21–30)
Median MUAC, mm (IQR)	266 (248–290)	268 (250–292)	268 (250–294)	268 (250–294)
Supplied ≥1 telephone contact	90% (2560/2840)	91% (2589/2837)	91% (2884/3184)	92% (2902/3160)
Resides in BHP HDSS	24% (690/2840)	24% (667/2837)	19% (595/3184)	18% (577/3160)
Resides within Bissau city	89% (2535/2835)	90% (2556/2833)	87% (2775/3182)	87% (2745/3160)
Infant characteristics				
Median age at inclusion, d (IQR)	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)
Admitted at hospital before inclusion	1% (32/2840)	1% (23/2837)	3% (81/3184)	2% (53/3160)
Median birthweight, g (IQR)	3100 (2800–3410)	3110 (2820–3400)	3120 (2800–3420)	3120 (2810–3420)
Median inclusion weight, g (IQR)	2970 (2700–3260)	2980 (2690–3250)	2990 (2700–3280)	3000 (2710–3280)
Male sex	52% (1470/2840)	52% (1477/2837)	52% (1643/3184)	52% (1630/3160)
Twin proportion	3% (98/2840)	3% (83/2837)	4% (133/3184)	3% (97/3160)
Mean size of postvaccination wheal, mm (SD)	4 (0.7)	4 (0.7)	4 (0.7)	4 (0.7)

Data are presented as % (n/N) unless otherwise indicated.
Abbreviations: BCG, Bacille Calmette-Guérin; BHP, Bandim Health Project; HDSS, Health and Demographic Surveillance System; IQR, interquartile range; MUAC, mid-upper-arm circumference; SD, standard deviation.
[a]Maternal BCG scar: data collection initiated on 11 July 2015 after 1244 study inclusions.

using the ballpoint pen method, and monitor mortality, morbidity, and prevalence of lymphadenitis (Supplementary Figure 4*A* and 4*B*).

Outcomes

The primary outcome was all-cause hospital admission at HNSM within 6 weeks after birth. Secondary outcomes were neonatal admissions, mortality by 6 weeks, BCG skin reaction frequency, PPD reactivity, and adverse events (left axillary lymphadenopathy, defined as a palpable lymph node with a diameter >1 cm).

Sample Size and Statistical Analyses

The target sample size of 12 000 neonates to test whether BCG-Denmark would be associated with fewer hospital admissions than BCG-Russia was based on the a priori hypothesis that there would be 258 six-week admissions in total, providing 80% power with an $\alpha = .05$ to show a 30% difference in overall admission incidence. Incidence rate ratios (IRRs) of hospital admission events comparing randomization groups were estimated in the recurrent-event Andersen-Gill Cox proportional hazards model with robust standard errors and age as the underlying time variable. Age was thus inherently controlled for. Person-years of risk were calculated from enrollment (day of randomization). Infants were not considered at risk of admission while admitted; days admitted thus did not contribute to the person-years of risk. We computed cumulative recurrent-event admission curves using the Kaplan-Meier estimate based on the date of admission. In-hospital case-fatality risk ratios (RRs) are reported as cohort study RRs assessed for significance using Fisher 2-sided exact test providing approximate CIs. We assessed effects on mortality in Cox models providing mortality rate ratios (MRRs) within the HDSS cohort, among infants with successful telephone follow-up, and for admitted non-HDSS infants not reached by telephone, and we present a combined mortality estimate from the 3 follow-up sources. Tests of proportionality of hazards were computed using Schoenfeld residuals.

Prevalence of a BCG reaction (defined as a scar, pustule, or papule) and a PPD response by strain were analyzed by binomial regression providing prevalence ratios (PRs). Prevalence of lymphadenitis by strain was assessed for significant using Fisher 2-sided exact test. We conducted 2 predefined additional analyses of the main outcome (Supplementary Appendix); stratification by season of enrollment (rainy season: June–November; dry season: December–May) and censoring from the first day of national OPV vaccination campaigns. We conducted 2 exploratory analyses (Supplementary Appendix) evaluating the 2-month prevalence of BCG skin pustules by randomization group using binomial regression and assessing the mean BCG skin reaction size using Student *t* test providing mean difference estimates by randomization group. A sensitivity analysis (Supplementary Appendix) with adjustment for preinclusion admissions and twinning was performed due to minor phase 2 inclusion imbalances. All analyses were per protocol and performed overall and by sex using StataIC version 12 software (StataCorp, College Station, Texas), and all estimates are reported with 95% CIs.

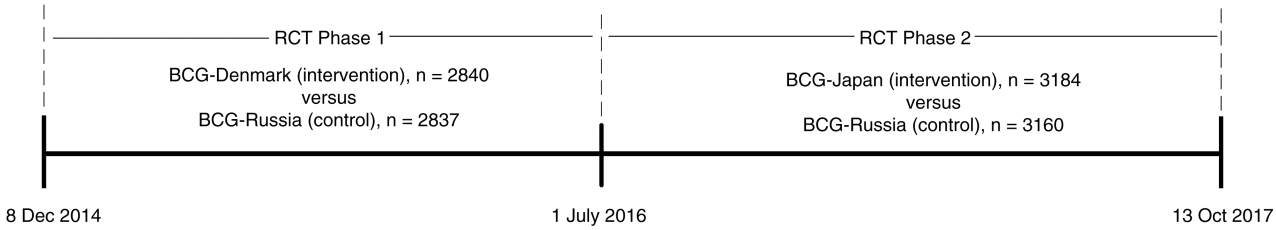

Figure 1. Periods of recruitment, by study phase. Abbreviations: BCG, Bacille Calmette-Guérin; RCT, randomized controlled trial.

Ethical Considerations

The study protocol was approved by the Guinea-Bissau Health Ministry's Research Coordination Committee (reference number 0020 CNES/INASA/2014) and given consultative approval by the Central Danish Ethical Committee (case number 1407397). The trial was conducted in accordance with the Helsinki Declaration ethical standards, and a data and safety monitoring board oversaw the trial. BHP provided free healthcare consultations and essential drugs to all infants invited to participate in the study, which was registered at ClinicalTrials.gov (NCT02447536).

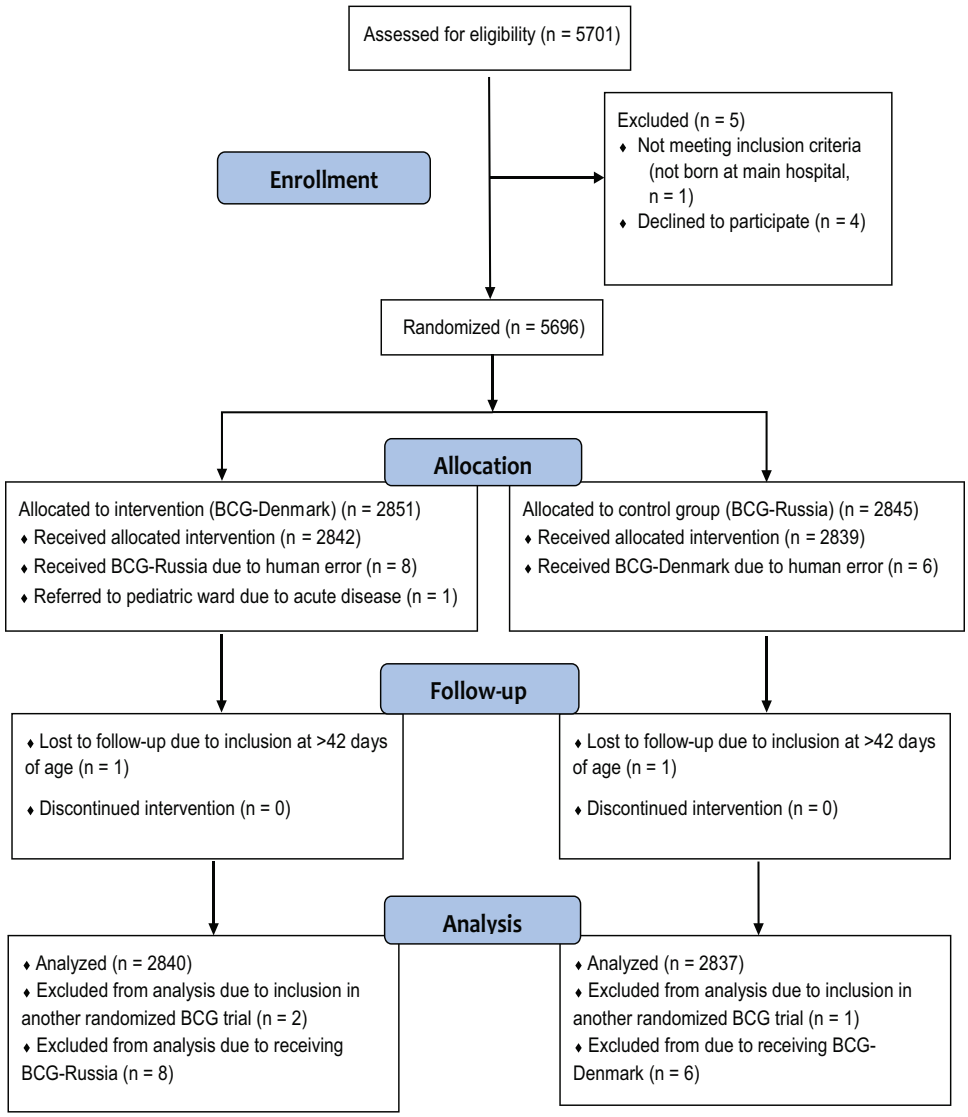

Figure 2. Consolidated Standards of Reporting Trials 2010 study flowchart, phase 1 comparing BCG-Denmark to BCG-Russia. Abbreviation: BCG, Bacille Calmette-Guérin.

RESULTS

Eligible participants were recruited from 8 December 2014 to 13 October 2017 (Figure 1). We assessed the eligibility of 12 298 neonates at the time of hospital discharge, of whom 224 infants could not be enrolled because they had not been born at HNSM and 17 mothers declined participation. We thus randomized 12 057 infants (phase 1: 5696; phase 2: 6361). Thirty-six neonates were excluded postenrollment owing to receipt of the wrong vaccine (n = 29), being enrolled in another RCT providing BCG to infants admitted to the nursery (n = 5), or being older than 6 weeks (n = 2). The trial cohort thus consisted of 12 021 infants (Table 1). Two neonates (phase 1: 1 BCG-Denmark; phase 2: 1 BCG-Russia) were randomized but not vaccinated since signs of disease were discovered postrandomization, and they were transferred to the pediatric ward where both died.

Phase 1 encompassed 5677 infants; 2840 received BCG-Denmark and 2837 received BCG-Russia (Figure 2). Baseline characteristics were evenly distributed between the randomization groups (Table 1). Phase 2 encompassed 6344 infants, of whom 3184 received BCG-Japan and 3160 received BCG-Russia (Figure 3). Compared to BCG-Russia, the BCG-Japan cohort had more children who had received preinclusion treatment at the pediatric ward (2.5% [81/3184] vs 1.7% [53/3160]; $P = .02$) and more twins (4.2% [133/3184] vs 3.1% [97/3160]; $P = .02$). The remaining baseline characteristics were evenly distributed (Table 1).

Morbidity by 6 Weeks of Age

There was a total of 616 registered admissions by 6 weeks, the overall admission incidence being 5.1% (616/12 021) (phase 1: 4.8% [270/5677]; phase 2: 5.5% [346/6344]). The overall CFR was 10.1% (phase 1: 15.2% [41/270]; phase 2: 6.1% [21/346]; Table 2).

In phase 1, the BCG-Denmark/BCG-Russia all-cause IRR was 1.08 (95% CI, .84–1.37; Supplementary Figure 5) and the case-fatality RR was 1.45 (95% CI, .81–2.59; Figure 4). By sex, the BCG-Denmark/BCG-Russia male IRR was 1.16 (95% CI, .84–1.60) vs 0.97 (95% CI, .67–1.41) for females (Supplementary Figure 6). In phase 2, the BCG-Japan/BCG-Russia all-cause IRR was 1.15 (95% CI, .93–1.43; Supplementary

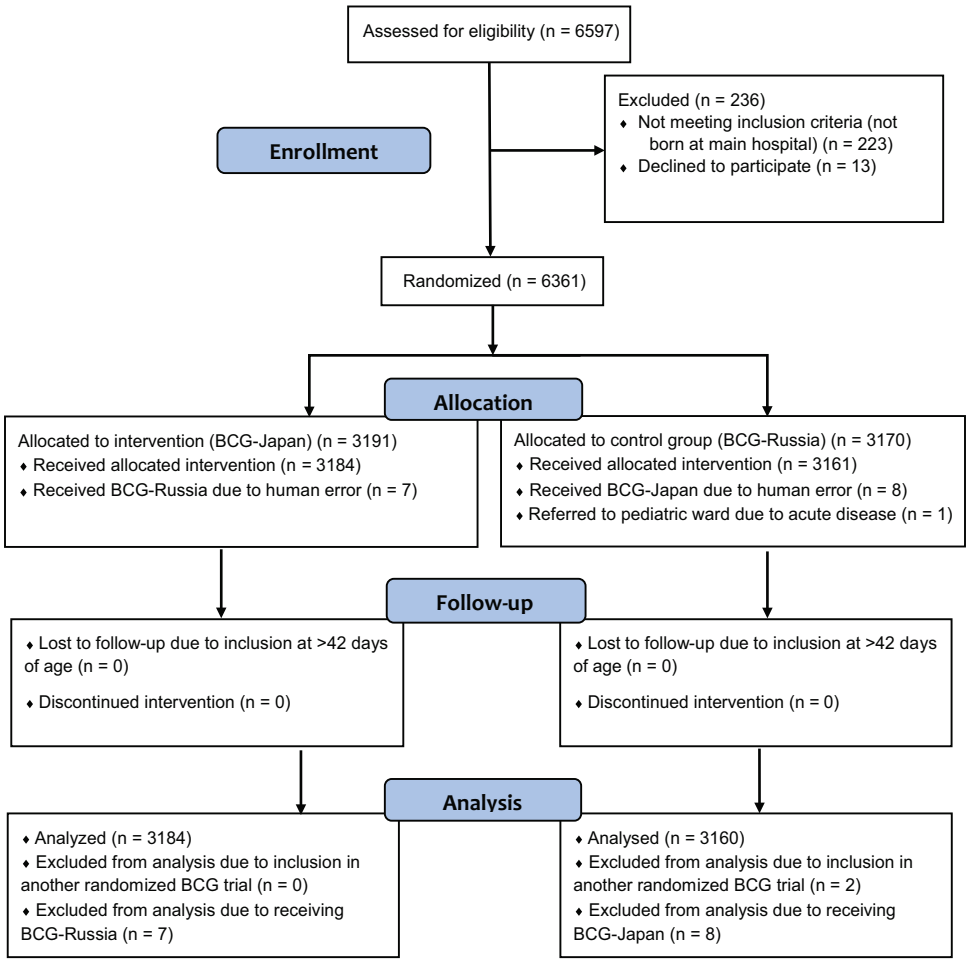

Figure 3. Consolidated Standards of Reporting Trials 2010 study flowchart, phase 2 comparing BCG-Japan to BCG-Russia. Abbreviation: BCG, Bacille Calmette-Guérin.

Table 2. Admission Incidence[a] and Case Fatality Within 6 Weeks of Age, by BCG Strain and Sex

	Denmark (n = 2840)		Russia (n = 2837)				Japan (n = 3184)		Russia (n = 3160)			
Sex	Admissions (Fatal)	Admission Rate per PY (Total PY)	Admissions (Fatal)	Admission Rate per PY (Total PY)	Denmark/Russia Admission IRR (95% CI)[b]	Denmark/Russia Case-Fatality RR (95% CI)[c]	Admissions (Fatal)	Admission Rate per PY (Total PY)	Admissions (Fatal)	Admission Rate per PY (Total PY)	Japan/Russia Admission IRR (95% CI)[b]	Japan/Russia Case-Fatality RR (95% CI)[c]
Infectious diseases												
Male	74 (7)	0.46 (162)	63 (9)	0.39 (164)	1.18 (.84–1.67)	0.66 (.26–1.68)	99 (5)	0.55 (181)	78 (5)	0.43 (180)	1.28 (.95–1.73)	0.79 (.24–2.63)
Female	47 (13)	0.31 (152)	51 (6)	0.34 (151)	0.91 (.61–1.36)	2.35 (.97–5.68)	66 (2)	0.39 (171)	66 (4)	0.39 (169)	1.00 (.70–1.41)	0.50 (.09–2.64)
Total	121 (20)	0.39 (314)	114 (15)	0.36 (314)	1.06 (.82–1.37)	1.26 (.68–2.33)	165(7)	0.47 (352)	144(9)	0.41 (349)	1.15 (.92–1.44)	0.68 (.26–1.78)
Noninfectious diseases												
Male	10 (3)	0.06 (162)	10 (0)	0.06 (164)	1.01 (.42–2.42)	…	10 (3)	0.06 (181)	8 (1)	0.04 (180)	1.25 (.49–3.15)	2.40 (.30–18.9)
Female	9 (2)	0.06 (152)	6 (1)	0.04 (151)	1.48 (.53–4.16)	1.33 (.15–11.6)	10 (0)	0.06 (171)	9 (1)	0.05 (169)	1.11 (.45–2.72)	…
Total	19 (5)	0.06 (314)	16 (1)	0.05 (314)	1.19 (.61–2.30)	4.21 (.55–32.4)	20 (3)	0.06 (352)	17 (2)	0.05 (349)	1.17 (.62–2.24)	1.28 (.24–6.76)
Total admissions												
Male	84 (10)	0.52 (162)	73 (9)	0.45 (164)	1.16 (.84–1.60)	0.97 (.42–2.25)	109(8)	0.60 (181)	86(6)	0.48 (180)	1.28 (.96–1.71)	1.05 (.38–2.92)
Female	56 (15)	0.37 (152)	57 (7)	0.38 (151)	0.97 (.67–1.41)	2.18 (.96–4.94)	76 (2)	0.44 (171)	75 (5)	0.44 (169)	1.01 (.73–1.39)	0.39 (.08–1.97)
Total	140 (25)	0.45 (314)	130 (16)	0.41 (314)	1.08 (.84–1.37)	1.45 (.81–2.59)	185(10)	0.53 (352)	161(11)	0.46 (349)	1.15 (.93–1.43)	0.79 (.35–1.81)

Data are presented as no. (%) unless otherwise indicated.
Abbreviations: BCG, Bacille Calmette-Guérin; CI, confidence interval; IRR, incidence rate ratio; PY, person-years; RR, risk ratio.
[a]Repeated admissions account for 2.9% (18/616) of the total admissions by 6 weeks.
[b]Recurrent-event Andersen-Gill Cox proportional hazards model.
[c]Cohort study RR (Fisher 2-sided exact test).

Figure 5) and the case-fatality RR was 0.79 (95% CI, .35–1.81; Figure 4). By sex, the male IRR was 1.28 (95% CI, .96–1.71) vs 1.01 (95% CI, .73–1.39) for females (Supplementary Figure 6).

Mortality by 6 Weeks of Age

Telephone follow-up was successful for 79.3% (4501/5677) of the cohort in phase 1, and the BCG-Denmark/BCG-Russia

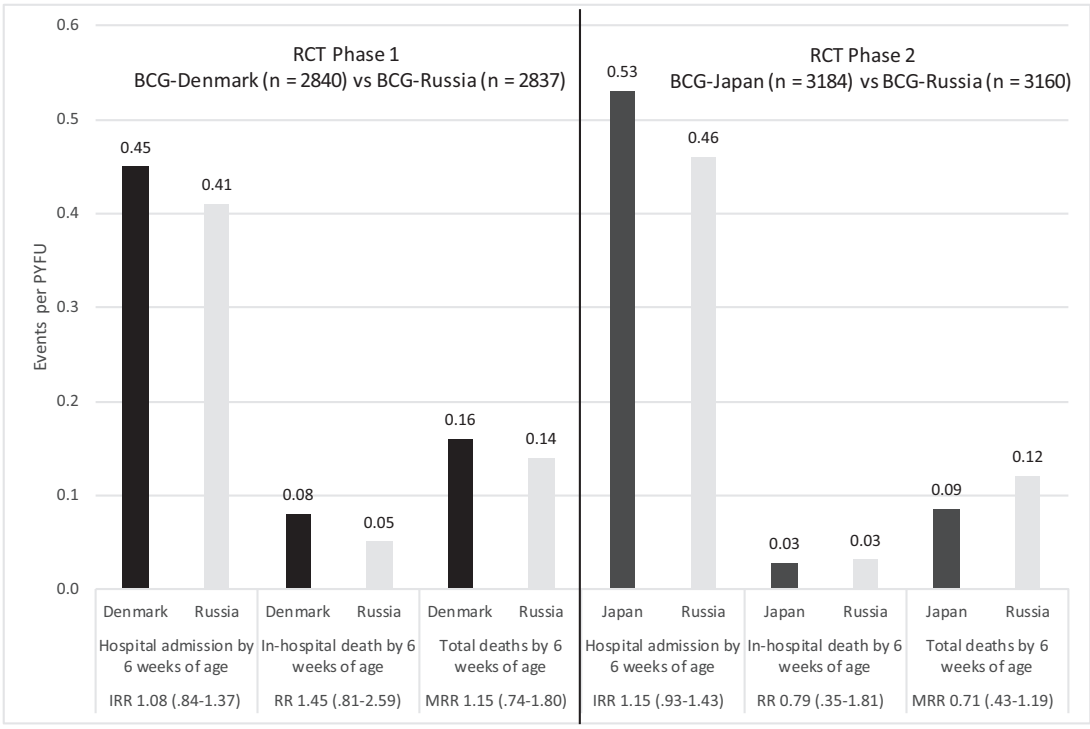

Figure 4. Hospital admissions, in-hospital mortality, and total deaths across the 2 trial phases within the first 6 weeks of life. IRRs estimated in recurrent event Andersen-Gill Cox proportional hazards models. Risk ratios estimated from cohort study RRs using Fisher exact test. MRRs estimated using Cox proportional hazards models with data from all 3 follow-up sources (telephone, Health and Demographic Surveillance System, and Simão Mendes National Hospital). Data in parentheses on the x-axis indicate the 95% confidence interval. Abbreviations: BCG, Bacille Calmette-Guérin; IRR, incidence rate ratio; MRR, mortality rate ratio; PYFU, person-years of follow-up; RCT, randomized controlled trial; RR, risk ratio.

Table 3. Study Deaths Within 6 Weeks of Age, by Strain of BCG, Sex, and Source of Follow-up

Sex	Mortality Rate by Age 6 wk per PY (No. of Deaths/Total PY)		Denmark/Russia 6-wk MRR (95% CI)[a]	Mortality Rate by Age 6 wk per PY (No. of Deaths/Total PY)		Japan/Russia 6-wk MRR (95% CI)[a]
	Denmark	Russia		Japan	Russia	
HDSS[b]						
Male	0.23 (9/39)	0.22 (8/37)	1.08 (.42–2.81)	0.09 (3/34)	0.13 (4/32)	0.71 (.16–3.18)
Female	0.20 (7/35)	0.11 (4/35)	1.67 (.49–5.72)	0.07 (2/31)	0.07 (2/30)	1.00 (.14–7.08)
Total	0.22 (16/74)	0.17 (12/72)	1.29 (.61–2.72)	0.08 (5/65)	0.10 (6/62)	0.81 (.25–2.65)
Telephone follow-up among non-HDSS residents[c]						
Male	0.15 (14/95)	0.11 (11/100)	1.32 (.60–2.92)	0.10 (12/116)	0.15 (18/118)	0.68 (.33–1.41)
Female	0.11 (10/91)	0.12 (11/91)	0.92 (.39–2.16)	0.05 (5/110)	0.08 (9/109)	0.56 (.19–1.66)
Total	0.13 (24/186)	0.12 (22/191)	1.12 (.63–2.00)	0.08 (17/226)	0.12 (27/227)	0.63 (.35–1.16)
In-hospital deaths among hospitalized non-HDSS residents not reached by telephone follow-up[d]						
Male	0.0 (0/0.3)	4.9 (1/0.2)	…	4.2 (2/0.5)	0.0 (0/0.4)	…
Female	4.1 (1/0.2)	2.3 (1/0.4)	1.66 (.09–29.0)	1.7 (1/0.6)	10.6 (2/0.2)	NA[e]
Total	2.0 (1/0.5)	3.1 (2/0.6)	0.62 (.05–7.15)	2.8 (3/1.1)	3.4 (2/0.6)	0.68 (.11–4.25)
Total deaths registered from HDSS, telephone, and hospital records[f]						
Male	0.17 (23/134)	0.15 (20/137)	1.17 (.64–2.13)	0.11 (17/151)	0.15 (22/150)	0.77 (.41–1.46)
Female	0.14 (18/126)	0.13 (16/127)	1.13 (.58–2.21)	0.06 (8/141)	0.09 (13/140)	0.61 (.25–1.47)
Total	0.16 (41/260)	0.14 (36/264)	1.15 (.74–1.80)	0.09 (25/292)	0.12 (35/290)	0.71 (.43–1.19)

Abbreviations: BCG, Bacille Calmette-Guérin; CI, confidence interval; HDSS, Health and Demographic Surveillance System; MRR, mortality rate ratio; NA, not applicable; PY, person-years.
[a]Cox proportional hazards model.
[b]Deaths recorded from all follow-up sources among residents in the HDSS (2529 infants, 21% of cohort). The following HDSS deaths occurred at the main hospital: phase 1, 54% (15/28) (BCG-Denmark: 50% [8/16]; BCG-Russia: 58% [7/12]). Phase 2, 18% (2/11) (BCG-Japan: 20% [1/5]; BCG-Russia: 17% [1/6]).
[c]Deaths recorded by telephone follow-up among non-HDSS infants with successful telephone follow-up (7467 infants, 62% of cohort). For non-HDSS deaths recorded by telephone, the following occurred at the main hospital: phase 1, 48% (22/46) (BCG-Denmark: 63% [15/24]; BCG-Russia: 32% [7/22]). Phase 2, 27% (12/44) (BCG-Japan: 29% [5/17]; BCG-Russia: 26% [7/27]).
[d]Among 2025 infants (17% of cohort) not reached by home visits or telephone follow-up, we identified 73 six-week hospital admissions, among which 8 infants died at the hospital.
[e]Not applicable due to too short follow-up time.
[f]For this estimate, HDSS data (2529 infants) were regarded as most precise. If a child was not from the HDSS, infants with successful telephone follow-up were included in the analysis (7467 infants), and if neither was available (2025 infants), admission data from the main hospital (73 admissions) were used to provide the combined MRR estimates.

MRR was 1.17 (95% CI, .73–1.88; Supplementary Table 1). For HDSS infants, the MRR was 1.29 (95% CI, .61–2.72) and the combined BCG-Denmark/BCG-Russia MRR estimate from the 3 follow-up sources (telephone, HDSS, HNSM) was 1.15 (95% CI, .74–1.80) (Table 3).

Table 4. Infants in the Health and Demographic Surveillance System Visited at Home by 2 Months of Age: BCG Reaction Prevalence and Size and Incidence of Lymphadenitis

Phase 1									
Sex	BCG Reaction Prevalence			Mean Size of BCG Reaction			Left Axillary Lymphadenitis[a]		
	% (n/N)		Denmark/Russia PR (95% CI)[b]	Size, mm (SD)		Reaction Size MD (95% CI)[c]	% (n/N)		Denmark/Russia PR (95% CI)
	Denmark	Russia		Denmark	Russia		Denmark	Russia	
Male	100 (280/280)	96 (256/267)	1.09[d] (1.06–1.12)	5.3 (1.3)	4.4 (1.2)	0.85[d] (.64–1.06)	0.3 (1/280)	0.0 (0/271)	…
Female	99 (252/255)	96 (235/246)	1.03[e] (1.00–1.07)	5.0 (1.3)	4.3 (1.1)	0.77[d] (.56–.99)	0.8 (2/260)	0.4 (1/252)	1.94 (.18–21.2)
Total	99 (532/535)	96 (491/513)	1.07[d] (1.05–1.09)	5.2 (1.3)	4.3 (1.2)	0.82[d] (.67–.96)	0.6 (3/540)	0.2 (1/523)	2.91 (.30–27.8)
Phase 2									
Sex	BCG Reaction Prevalence			Mean Size of BCG Reaction			Left Axillary Lymphadenitis		
	% (n/N)		Japan/Russia PR (95% CI)	Size, mm (SD)		Reaction Size MD (95% CI)	% (n/N)		Japan/Russia PR (95% CI)
	Japan	Russia		Japan	Russia		Japan	Russia	
Male	98 (254/259)	96 (212/222)	1.03 (.99–1.06)	5.5 (1.3)	4.7 (1.2)	0.80[d] (.57–1.03)	0.8 (2/260)	0.0 (0/222)	…
Female	97 (211/218)	96 (221/230)	1.01 (.97–1.04)	5.3 (1.8)	4.7 (1.2)	0.61[d] (.33–.89)	0.5 (1/218)	0.0 (0/230)	…
Total	98 (465/477)	96 (433/452)	1.02 (.99–1.04)	5.4 (1.5)	4.7 (1.2)	0.71[d] (.53–.90)	0.6 (3/478)	0.0 (0/452)	…

Abbreviations: BCG, Bacille Calmette-Guérin; CI, confidence interval; MD, mean difference; PR, prevalence ratio; SD, standard deviation.
[a]History of left axillary lymphadenitis reported by the mother or an enlarged lymph node at physical examination at the 2-month visit.
[b]Binomial regression.
[c]Student *t* test providing estimates of MD in BCG reaction size (height + width / 2) between intervention and control group.
[d]$P < .001$.
[e]$P < .05$.

In phase 2, telephone follow-up was successful for 79.9% (5066/6344) of the cohort and the Japan/Russia MRR estimate was 0.66 (95% CI, .38–1.15; Supplementary Table 1), 0.81 (95% CI, .25–2.65) for HDSS infants, and 0.71 (95% CI, 0.43–1.19) in the combined (telephone, HDSS, HNSM) estimate (Table 3).

BCG Skin Reactions

Both BCG-Denmark and BCG-Japan produced more and larger local skin reactions than BCG-Russia (Supplementary Figure 7). By 2 months, 99.4% (532/535) of infants inoculated with BCG-Denmark had developed a reaction vs 95.7% (491/513) for BCG-Russia (PR, 1.07 [95% CI, 1.05–1.09]). For BCG-Japan, 97.5% (465/477) of infants had a reaction vs 95.8% (433/452) for BCG-Russia (PR, 1.02 [95% CI, .99–1.04]; Table 4). By 6 months, the Denmark/Russia skin reaction PR was 1.07 (95% CI, 1.04–1.10; Table 5) and the Japan/Russia PR was 1.06 (95% CI, 1.02–1.09; Table 6).

PPD Responses

Two PPD preparations of strengths 2 and 10 tuberculosis units (TU) were administered in phase 1 (Supplementary Figure 8), and BCG-Denmark induced superior reactivity to both. Among infants given PPD 2 TU, the reaction prevalence was 34.9% (23/66) for BCG-Denmark vs 10.9% (7/64) for BCG-Russia, the BCG-Denmark/BCG-Russia PR being 3.19 (95% CI, 1.47–6.90; Table 5). For infants given PPD 10 TU, the reaction prevalence was 64.3% (189/294) for BCG-Denmark vs 55.5% (172/310) for BCG-Russia (PR, 1.16 [95% CI, 1.02–1.32]; Table 5).

The BCG-Japan PPD 2 TU reaction prevalence was 25.8% (85/329) vs 14.1% (47/334) for BCG-Russia (PR, 1.84 [95% CI, 1.33–2.53]; Table 6).

Adverse Events

Surveillance of lymphadenitis prevalence revealed an overall 6-month incidence of 0.6% (13/2167) for HDSS infants. In phase 1, the incidence was 1.0% (6/582) for BCG-Denmark vs 0.2% (1/575) for BCG-Russia (P = .12, 2-sided Fisher exact test). In phase 2, the incidence was 1.2% (6/519) for BCG-Japan and 0.0% (0/491) for BCG-Russia (P = .03, 2-sided Fisher exact test) (Table 6 and Figure 5). All infants were unaffected by the lymphadenitis, of which none were suppurative.

Prespecified analyses stratified by sex did not reveal marked sex-differential BCG strain effects (Tables 2–5 and Supplementary Tables 1–4).

DISCUSSION

The present RCT is the first to compare major BCG strains head-to-head for both their specific immunogenicity and their NSEs on overall infant health. We found no effects of BCG strains on all-cause admission incidence, but a tendency toward fewer deaths in the BCG-Japan cohort that warrants further investigation. Both BCG-Denmark and BCG-Japan induced

Table 5. Infants in the Health and Demographic Surveillance System Visited at Home by 6 Months of Age: BCG Reaction Prevalence and Size, Purified Protein Derivative Responses, and Incidence of Lymphadenitis (Phase 1)

	Phase 1											
	BCG Reaction Prevalence			Mean Size of BCG Reaction			Prevalence of Reactivity to PPD 2 TU[a]			Prevalence of Reactivity to PPD 10 TU[a]			Left Axillary Lymphadenitis[b]	
	% (n/N)		Denmark/Russia	Size, mm (SD)		Reaction Size	% (n/N)		Denmark/	% (n/N)		Denmark/	% (n/N)	
Sex	Denmark	Russia	PR (95% CI)[c]	Denmark	Russia	MD (95% CI)[d]	Denmark	Russia	Russia PR (95% CI)[c]	Denmark	Russia	Russia PR (95% CI)[c]	Denmark	Russia
Male	99 (223/225)	93 (199/215)	1.09[e] (1.05–1.13)	4.9 (1.3)	4.0 (1.3)	0.89[e] (.63–1.15)	38 (15/40)	13 (4/12)	3.00[f] (1.10–8.16)	63 (94/149)	56 (86/155)	1.14 (.94–1.37)	0.4 (1/245)	0.0 (0/238)
Female	99 (196/199)	93 (183/197)	1.06[e] (1.02–1.11)	4.7 (1.1)	3.8 (1.1)	0.82[e] (.60–1.04)	31 (8/26)	9 (3/32)	3.28 (.97–11.1)	66 (95/145)	56 (86/155)	1.18 (.98–1.42)	0.9 (2/223)	0.0 (0/227)
Total	99 (419/424)	93 (382/412)	1.07[e] (1.04–1.10)	4.8 (1.2)	3.9 (1.2)	0.86[e] (.69–1.03)	35 (23/66)	11 (7/64)	3.19[g] (1.47–6.90)	64 (189/294)	56 (172/310)	1.16[f] (1.02–1.32)	0.6 (3/465)	0.0 (0/465)

Abbreviations: BCG, Bacille Calmette-Guérin; CI, confidence interval; MD, mean difference; PPD, purified protein derivative; PR, prevalence ratio; SD, standard deviation; TU, tuberculosis units.

[a]Prevalence of a positive PPD reaction response defined as >5 mm in size for both 2 TU and 10 TU as specified by the manufacturer, Statens Serum Institut.

[b]History of left axillary lymphadenitis reported by the mother or an enlarged lymph node at physical examination at a home visit. None of the cases identified at 6 months had been identified at 2 months; thus, the total number of left axillary lymphadenitis was 13 cases (7 identified at age 2 months and 6 identified at age 6 months).

[c]Binomial regression.

[d]Student t test providing estimates of MD in BCG reaction size (height + width / 2) between intervention and control group.

[e]$P < .001$.

[f]$P < .05$.

[g]$P < .01$.

Table 6. Infants in the Health and Demographic Surveillance System Visited at Home by 6 Months of Age: BCG Reaction Prevalence and Size, Purified Protein Derivative Responses, and Incidence of Lymphadenitis (Phase 2)

Phase 2										
Sex	BCG Reaction Prevalence			Mean Size of BCG Reaction			Prevalence of Reactivity to PPD 2 TU[a]			Left Axillary Lymphadenitis[b]
	% (n/N)		Japan/Russia PR (95% CI)[c]	Size, mm (SD)		Reaction Size MD (95% CI)[d]	% (n/N)		Japan/Russia PR (95% CI)[c]	% (n/N)
	Japan	Russia		Japan	Russia		Japan	Russia		Japan	Russia
Male	98 (193/198)	93 (167/179)	1.04 (1.00–1.09)	4.7 (1.3)	4.3 (1.3)	0.41[e] (.14–.68)	26 (46/175)	15 (26/169)	1.71[f] (1.11–2.63)	0.9 (2/216)	0.0 (0/203)
Female	96 (163/170)	90 (163/181)	1.06[f] (1.01–1.13)	4.6 (1.3)	4.0 (1.2)	0.58[g] (.30–.86)	25 (39/154)	13 (21/165)	1.99[g] (1.23–3.23)	0.5 (1/193)	0.0 (0/192)
Total	97 (356/368)	92 (330/360)	1.06[e] (1.02–1.09)	4.6 (1.3)	4.1 (1.3)	0.50[g] (.30–.69)	26 (85/329)	14 (47/334)	1.84[g] (1.33–2.53)	0.7 (3/409)	0.0 (0/395)

Abbreviations: BCG, Bacille Calmette-Guérin; CI, confidence interval; MD, mean difference; PPD, purified protein derivative; PR, prevalence ratio; SD, standard deviation; TU, tuberculosis units.

[a]Prevalence of a positive PPD reaction response defined as >5 mm in size for both 2 TU and 10 TU as specified by the manufacturer, Statens Serum Institut.

[b]History of left axillary lymphadenitis reported by the mother or an enlarged lymph node at physical examination at a home visit. None of the cases identified at 6 months had been identified at 2 months; thus, the total number of left axillary lymphadenitis was 13 cases (7 identified at age 2 months and 6 identified at age 6 months).

[c]Binomial regression.

[d]Student *t* test providing estimates of MD in BCG reaction size (height + width / 2) between intervention and control group.

[e]$P < .01$.

[f]$P < .05$.

[g]$P < .001$.

better immune responses (PPD reactivity, BCG reactions) and more lymphadenitis than BCG-Russia.

Strengths and Weaknesses

The RCT design inherently carries a low risk of bias, and the large-scale RCT had balanced randomization between the intervention and control groups, with the exception that more phase 2 infants randomized to BCG-Japan were same-sex twins and more had received preinclusion hospital treatment. The BCG-Japan cohort could thus have been weaker than the control group, but adjustment did not alter estimates. Almost all neonates were vaccinated within the first days of life, and any interference on the effects of BCG from prevaccination exposure to environmental mycobacteria would be minimal.

It is a limitation that the intervention was changed during the RCT and the results should be interpreted in that light. The admission rate was higher than we had anticipated: We registered 270 admissions in phase 1 and 346 in phase 2, and both comparisons thus had >80% power to show a ≥30% reduction in admission incidence associated with any of the strains.

At the HNSM pediatric ward, diagnoses are mainly based on clinical presentation due to scarce resources. By the end of phase 1 of the RCT, however, Médecins Sans Frontières implemented major structural changes to the ward. Standards of care were improved with emphasis on lowering the sepsis mortality by rigorous early treatment of suspected cases. The changes can have affected both hospital triage procedures and the in-hospital case fatality. It is plausible that this has reduced mortality, especially among the frailer infants affected by severe infection. In phase 2, there was indeed more early-life admissions and fewer hospital deaths, primarily due to a large drop in the in-hospital sepsis case-fatality rate.

We assessed mortality via home visits for the HDSS cohort, from telephone follow-up data, and data on admitted infants. While the 3 assessments had different strengths and limitations with respect to the quality and completeness of data, there was a fair degree of consistency of MRR estimates across the data sources.

Interpretation

The marked interstrain differences affecting BCG reactions, PPD reactivity, and adverse events confirmed our hypothesis that the BCG-Denmark and BCG-Japan strains are more immunogenic than BCG-Russia. More immunogenic vaccines are likely to provide better protection against TB [19]. It must be stressed, however, that the importance of a positive PPD reaction and BCG scar prevalence for protection against TB is debated [20].

In 2 studies evaluating immunogenicity, the strains best at producing BCG reactions also induced the highest TB-specific cytokine responses and polyfunctional CD4 T-cell proportions in humans [8, 21]. In parallel, fewer BCG reactions and less

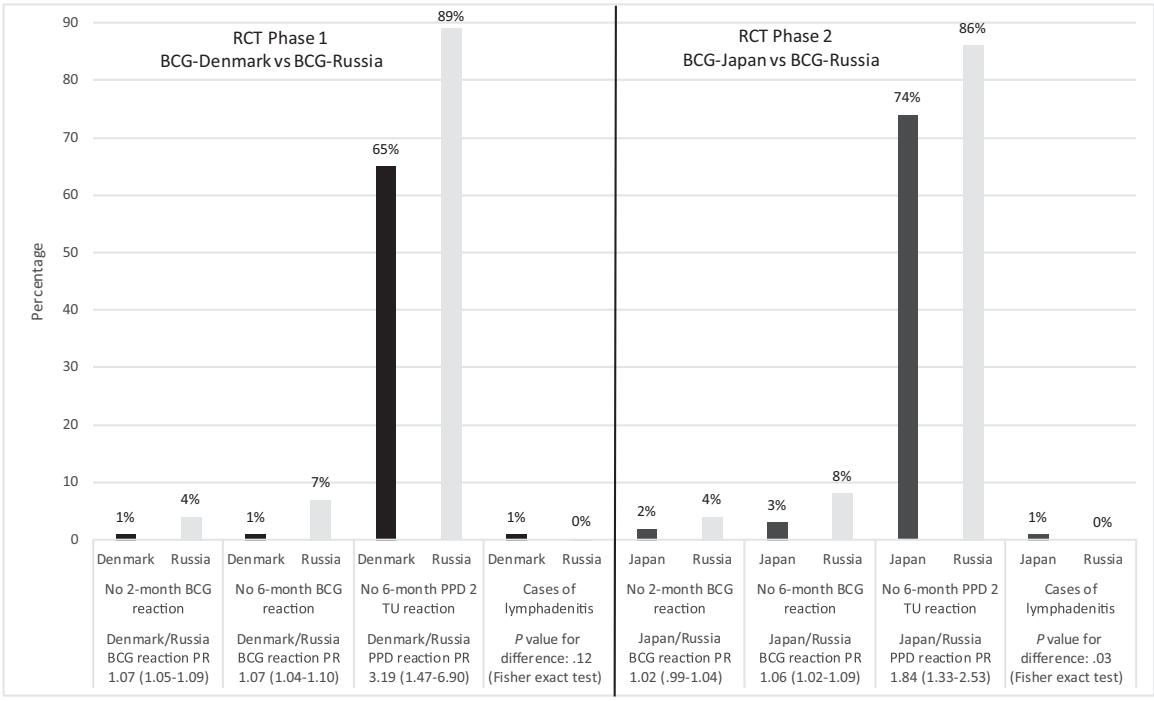

Figure 5. Percentage of infants in the Health and Demographic Surveillance System presenting no BCG reaction by 2 and 6 months of age, no PPD reaction, and cases of lymphadenitis by 6 months of age. BCG and PPD reaction prevalence ratios estimated using binomial regression. Prevalence of lymphadenitis by strain tested with Fisher exact test providing approximate 95% confidence intervals. Abbreviations: BCG, Bacille Calmette-Guérin; PPD, purified protein derivative; PR, prevalence ratio; RCT, randomized controlled trial; TU, tuberculosis units.

PPD reactivity is associated with lower mycobacterial vaccine viability and reduced immunogenic potency in animals [22]. It is unclear whether the specific and nonspecific effects of BCG are mutually correlated to the potency of the BCG strain, but having a BCG scar and a positive PPD response is strongly associated with lower all-cause mortality [12–17].

Our BCG skin reaction rates were high also for BCG-Russia; previous studies have reported BCG-Russia reaction rates ranging from 87% in urban Bissau and 83% in a small RCT from Australia, to as low as 52% in rural Guinea-Bissau and Uganda [8, 16, 21, 23]. All of our 12 000 vaccinations were performed by just 2 specialized, experienced vaccinators and we measured large postvaccination wheals, which is an important indicator of vaccination quality [10]. Given the importance of BCG scarring and PPD reactivity for overall mortality, our evaluation of strain effects on mortality might be conservative due to the high reaction rate in the BCG-Russia group.

Studies have shown marked differences in the contents of viable mycobacteria in BCG vaccines, with BCG-Japan holding the highest percentage of live bacteria upon reconstitution [24, 25]. Probably due to the quantity of intradermally deposited CFUs, there is a clear association between the postvaccination wheal size and the subsequent BCG reaction prevalence and PPD reactivity [10, 11]. In settings with less-experienced vaccinators and thus more frequent spills or inadequately delivered doses, strain-related BCG skin reaction rate differences could become more evident, as might be reflected in the studies mentioned above. The increased incidence of lymphadenitis that we report for BCG-Denmark and BCG-Japan is the downside to the use of immunogenic strains, but we witnessed no cases of severe lymphadenitis, which is also related to poor vaccination technique.

Perspectives

Many novel TB vaccine candidates under development are either subunit/booster vaccines designed to supplement BCG or recombinant BCG vaccines. Novel TB vaccines that reach phase 3 testing are likely to be tested against BCG to demonstrate superior efficacy and safety, and both the protective effects against TB and NSEs should be tested against an efficacious strain.

Even minor strain differences carry great importance for the >100 million infants that are BCG-vaccinated yearly. Updating the global strain policy could boost the beneficial NSEs of BCG and if protection against TB could be improved by just 1%, we would prevent an estimated 83 000 TB cases and 18 000 TB deaths [20].

The beneficial effects of BCG on overall survival are likely due to training and epigenetic reprogramming of the immune system, providing protection against BCG-unrelated pathogens [4]. Large-scale BCG vaccination is feasible and will remain a cornerstone in global anti-TB efforts for decades to come. Identifying the most efficient BCG strain is a low-hanging fruit with profound implications for the global infectious disease

burden, but no global policy has been formulated yet due to the striking scarcity of studies [26]. To that end, the present study unequivocally documents that BCG-Denmark and BCG-Japan induce more BCG reactions and PPD responses than BCG-Russia.

In conclusion, the BCG strains were not associated with differences in hospital admission incidence. BCG-Denmark and BCG-Japan were more immunogenic than BCG-Russia by the measures traditionally viewed as surrogates for successful immunization.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. C. S. B. and P. A. were the principal investigators and guarantors. C. S. B., F. S.-B., M. B.-A., and P. A. designed and initiated the trial. C. G., E. B. S., F. S.-B., and I. M. were responsible for the recruitment and follow-up of participants. F. S.-B. wrote the first draft of the manuscript. C. B. Ø. and F. S.-B. were responsible for the statistical analyses. All authors contributed to and approved the final manuscript. All authors had full access to all study data and bear the responsibility for their analysis and the decision to submit for publication.

Acknowledgments. The authors thank the mothers and infants who participated in the study; study supervisors Gabriel Marciano Gomes, Odete Correia, and Paulo Umbasse for their conscientious efforts in the data collection for the study; the members of the data safety and monitoring board (Robin Bailey, Poul-Erik Kofoed, and Andreas Kryger Jensen); and freelance photographer Sofia Busk for allowing them to include her photos in this publication (Supplementary Figures 1–3, 4*A*, *B*). The authors also thank Anders Rehfeld, Anton Pottegård, and Mike Berendsen for providing a presubmission review of the manuscript. Deidentified participant data with a data dictionary can be shared after approval of a data-sharing proposal sent to C. S. B. (cb@ssi.dk).

Disclaimer. The funding agencies had no influence on the study design, data collection, analysis, interpretation or writing of the manuscript, or the decision to submit this work for publication. Statens Serum Institut (SSI) is a producer of BCG, Bacille Calmette-Guérin. However, SSI did not fund the vaccines, the study, or the researchers and did not have any influence on the study design, data collection, analysis, interpretation or writing of the report, nor the decision to submit the manuscript for publication.

Financial support. This work was supported by the Danish National Research Foundation (DNRF) (grant number DNRF108). DNRF and the University of Southern Denmark (grant reference HNP) funded the PhD scholarship to F. S.-B. The Bandim Health Project has previously received support by the Danish International Development Agency.

Potential conflicts of interest. Several of the authors (F. S.-B., P. A., and C. S. B.) are affiliated with the SSI in Copenhagen, which administers, but does not finance, their grants. All other authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Luca S, Mihaescu T. History of BCG vaccine. Maedica (Buchar) **2013**; 8:53–8.
2. Mangtani P, Abubakar I, Ariti C, et al. Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials. Clin Infect Dis **2014**; 58:470–80.
3. Biering-Sørensen S, Aaby P, Lund N, et al. Early BCG-Denmark and neonatal mortality among infants weighing <2500 g: a randomized controlled trial. Clin Infect Dis **2017**; 65:1183–90.
4. Benn CS, Netea MG, Selin LK, Aaby P. A small jab—a big effect: nonspecific immunomodulation by vaccines. Trends Immunol **2013**; 34:431–9.
5. Netea MG, Joosten LAB, Latz E, et al. Trained immunity: a program of innate immune memory in health and disease. Science **2016**; 352:aaf1098.
6. Zhang W, Zhang Y, Zheng H, et al. Genome sequencing and analysis of BCG vaccine strains. PLoS One **2013**; 8:e71243.
7. Jayaraman K, Adhisivam B, Nallasivan S, et al. Two randomized trials of the effect of the Russian strain of bacillus Calmette-Guerin alone or with oral polio vaccine on neonatal mortality in infants weighing <2000 g in India. Pediatr Infect Dis J **2019**; 38:198–202.
8. Anderson EJ, Webb EL, Mawa PA, et al. The influence of BCG vaccine strain on mycobacteria-specific and non-specific immune responses in a prospective cohort of infants in Uganda. Vaccine **2012**; 30:2083–9.
9. Funch KM, Thysen SM, Rodrigues A, et al. Determinants of BCG scarification among children in rural Guinea-Bissau: a prospective cohort study. Hum Vaccin Immunother **2018**; 14:2434–42.
10. Roth A, Sodemann M, Jensen H, et al. Vaccination technique, PPD reaction and BCG scarring in a cohort of children born in Guinea-Bissau 2000–2002. Vaccine **2005**; 23:3991–8.
11. Guérin N, Teulières L, Noba A, Schlumberger M, Bregère P, Chauvin P. Comparison of the safety and immunogenicity of the lyophilized Mérieux seed and the World Health Organization working reference BCG vaccines in school-aged children in Senegal. Vaccine **1999**; 17:105–9.
12. Garly ML, Martins CL, Balé C, et al. BCG scar and positive tuberculin reaction associated with reduced child mortality in West Africa. A non-specific beneficial effect of BCG? Vaccine **2003**; 21:2782–90.
13. Roth A, Gustafson P, Nhaga A, et al. BCG vaccination scar associated with better childhood survival in Guinea-Bissau. Int J Epidemiol **2005**; 34:540–7.
14. Roth A, Sodemann M, Jensen H, et al. Tuberculin reaction, BCG scar, and lower female mortality. Epidemiology **2006**; 17:562–8.
15. Timmermann CAG, Biering-Sørensen S, Aaby P, et al. Tuberculin reaction and BCG scar: association with infant mortality. Trop Med Int Health **2015**; 20:1733–44.
16. Storgaard L, Rodrigues A, Martins C, et al. Development of BCG scar and subsequent morbidity and mortality in rural Guinea-Bissau. Clin Infect Dis **2015**; 61:950–9.
17. Berendsen MLT, Øland CB, Bles P, et al. Maternal priming: bacillus Calmette-Guérin (BCG) vaccine scarring in mothers enhances the survival of their child with a BCG vaccine scar. J Pediatr Infect Dis Soc **2019.** doi:10.1093/jpids/piy142.
18. Schaltz-Buchholzer F, Biering-Sørensen S, Lund N, et al. Early BCG vaccination, hospitalizations, and hospital deaths: analysis of a secondary outcome in 3 randomized trials from Guinea-Bissau. J Infect Dis **2019**; 219:624–32.
19. Zhang L, Ru HW, Chen FZ, et al. Variable virulence and efficacy of BCG vaccine strains in mice and correlation with genome polymorphisms. Mol Ther **2016**; 24:398–405.
20. Ritz N, Hanekom WA, Robins-Browne R, Britton WJ, Curtis N. Influence of BCG vaccine strain on the immune response and protection against tuberculosis. FEMS Microbiol Rev **2008**; 32:821–41.
21. Ritz N, Dutta B, Donath S, et al. The influence of bacille Calmette-Guerin vaccine strain on the immune response against tuberculosis: a randomized trial. Am J Respir Crit Care Med **2012**; 185:213–22.
22. Vallishayee RS, Shashidhara AN, Bunch-Christensen K, Guld J. Tuberculin sensitivity and skin lesions in children after vaccination with 11 different BCG strains. Bull World Health Organ **1974**; 51:489–94.
23. Frankel H, Byberg S, Bjerregaard-Andersen M, et al. Different effects of BCG strains—a natural experiment evaluating the impact of the Danish and the Russian BCG strains on morbidity and scar formation in Guinea-Bissau. Vaccine **2016**; 34:4586–93.
24. World Health Organization, Expert Committee on Biological Standardization. WHO Technical Report Series, No. 977: 60th report (19–23 October 2009). Available at: http://apps.who.int/medicinedocs/en/m/abstract/Js21091en/. Accessed 14 April 2019.
25. Behr MA. BCG—different strains, different vaccines? Lancet Infect Dis **2002**; 2:86–92.
26. Shann F. Substantial benefits from finding the most effective BCG strain. Lancet Respir Med **2016**; 4:e35.